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Porcine *NAMPT* gene: search for polymorphism, mapping and association studies

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Summary

NAMPT encodes an enzyme catalysing the rate-limiting step in NAD biosynthesis. The extracellular form of the enzyme is known as adipokine visfatin. We detected SNP AM999341:g.669T>C (referred to as *669T>C*) in intron 9 and SNP FN392209:g.358A>G (referred to as *358A>G*) in the promoter of the gene. RH mapping linked the gene to microsatellite *SW944*. Linkage analysis placed the gene on the current USDA – USMARC linkage map at position 92 cM on SSC9. Association analyses were performed in a wild boar × Meishan F_2 family (W × M), with 45 traits recorded (growth and fattening, fat deposition, muscling, meat quality, stress resistance and other traits), and in a commercial Landrace × Chinese-European (LCE) synthetic population with records for 15 traits (growth, fat deposition, muscling, intramuscular fat, meat colour and backfat fatty acid content). In the W × M, SNP 669T>C was associated with muscling, fat deposition, growth and fattening, meat quality and other traits and in the LCE with muscling, meat quality and backfat fatty acid composition. In the W × M, SNP 358A>G was associated with muscling, fat deposition, growth and other traits. After correction for multiple testing, the *NAMPT* haplotypes were associated in the W × M with, in descending order, muscling ($q = 0.0056$), growth ($q = 0.0056$), fat deposition ($q = 0.0109$), fat-to-meat ratio ($q = 0.0135$), cooling losses ($q = 0.0568$) and longissimus pH_U ($q = 0.0695$). The SNPs are hypothesized to be in linkage disequilibrium with a causative mutation affecting energy metabolism as a whole rather than fat metabolism alone.

Keywords association study, carcass composition, genetic mapping, meat quality, *NAMPT*, pig, polymorphism.

NAMPT encodes the enzyme nicotinamide 5-phosphoribosyl-1-pyrophosphate transferase (NAMPT, EC 2.4.2.12), which catalyses the rate-limiting step in NAD biosynthesis. In mammals, NAMPT has an intracellular form which functions as an NAD biosynthetic enzyme and an extracellular form, originally described as pre-B-cell colony-enhancing factor 1 – PBEF1 and later named visfatin. NAMPT is a novel adipocytokine secreted preferentially by visceral fat tissue, rather than subcutaneous fat, in both humans and mice (Imai 2009).

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In adult humans, plasma concentrations of visfatin were shown to be positively associated with obesity, although negative associations were also reported (Sommer *et al.* 2008). Associations between polymorphisms of human *NAMPT*, insulin levels and obesity were reported (Bailey *et al.* 2006; McKenzie 2008; Blakemore *et al.* 2009). Chen *et al.* (2007) and Palin *et al.* (2008) reported porcine mRNA sequences, RNA expression and splicing variants of *NAMPT*.

The aim of this work was to search for polymorphisms within the porcine *NAMPT* gene, to map the gene, and to perform association analyses between polymorphic markers and performance, carcass composition and meat quality traits.

We detected SNP AM999341:g.669T>C in intron 9 and SNP FN392209:g.358A>G in the promoter region 913 bp upstream from the translation initiation codon ATG (for

PCR amplification, sequencing and SNP detection see Appendix S1 and Table S1). Allele frequencies estimated in unrelated animals of seven breeds and European wild boar are given in Table S2. Radiation hybrid (RH) mapping on the IMpRH panel and linkage mapping on the USDA MARC backcross family were performed as described elsewhere (Čepica *et al.* 2006). RH mapping showed the most significant linkage to microsatellite *SW944* (retention 41%, 0.52 R, LOD = 7.53) located at position 83.3 cM on the USDA – USMARC linkage map of SSC9 (http://agp.gene.staff.or.jp/agp/db/linkage/linkage.html). Two-point linkage analysis based on 25 informative meioses assigned the *NAMPT* gene to *KS5* (θ = 0.00, LOD = 6.32). Multipoint linkage analysis placed the *NAMPT* gene on the current USDA – USMARC linkage map at position 92 cM on SSC9. Our results are in agreement with the previous location of the gene on SSC9 q23 (Nowacka-Woszuk *et al.* 2008).

For the association study, a European wild boar × Meishan (W × M) family was used with 45 traits recorded for carcass composition and meat quality listed in Table S3 (Müller *et al.* 2000; Geldermann *et al.* 2003). Pigs were slaughtered at the age of 210 ± 6 days and live weight of 71.7 ± 13.8 kg. The W × M family was used because of the great number of precisely measured traits despite a large extent of linkage disequilibria (LD) expected. Association analyses were validated in a commercial Landrace × Chinese-European (LCE) synthetic population with 15 trait records listed in Table 2 and described by Óvilo *et al.* (2006), slaughtered at 127 ± 11.6 kg and an average age of 198 days.

Associations between *NAMPT* genotypes/haplotypes and traits were analysed using the GLM procedure of SAS, release 8.2 (SAS Institute Inc.). In the W × M family, the statistical model included fixed effects of *NAMPT* genotype/haplotype, season (2-month interval), sex, litter number (one or two) and slaughter age as a continuous linear effect in the model (Appendix S1). Carcass weight was not used as covariate for fat deposition and muscling traits, as it would not reflect the biological reality as a result of extremely great variability of slaughter weight in F_2 animals (23–107 kg). Instead, traits already adjusted for body size (dressing A, dressing B) and fat-to-meat ratios were used. In the LCE, the statistical model included the genotype of *NAMPT* locus, the slaughter batch, and other covariates that depend on the analysed trait: age at slaughter for growth traits; carcass weight for backfat measures, weights of premium cuts and Minolta colour parameters; and mean of backfat measured at P2 and m. gluteus medius points for the fatty acid profile. The *P* values were adjusted for multiple testing according to Benjamini & Hochberg (1995). According to Lu *et al.* (2008), *q* values up to 0.20 could be considered significant.

In the W × M, SNP *669T>C* segregated in both wild boar and Meishan founder animals (for genotyping of the SNP see Appendix S1). This SNP was associated ($P < 0.05$, q = 0.0572–0.0846) with fat deposition, muscling, growth and fattening, meat quality and other traits, but not with fat-to-meat ratio (Table S4). Animals with *TT* genotype had lower values for the fat deposition and muscling, grew slowly, and had lower food consumption, lower liver weight and shorter carcasses when compared with animals with *TC* and *CC* genotypes. Animals with *TT* genotype had a higher weight of whole ham relative to half carcass weight (dressing B) than animals with TC genotype, while proportion of ham meat weight relative to half carcass weight (dressing A) was not significantly different. The *TT* animals had higher cooling loss and higher pH_U in *m. longissimus lumborum et thoracis* and *m. semimenbranosus*, when compared with animals with *TC* and *CC* genotypes. Variants in the *669T>C* locus explained up to 2.8% of total phenotypic variance (PV) in F_2 animals.

The SNP *358A>G* is located 913 bp upstream from the translation initiation codon ATG. We searched alleles of the locus for differences in transcription factor binding sites (TFBS) using TESS software (Schug & Overton 1997). The *G* allele has several TFBS that are missing in the *A* allele, particularly TFBS for *HNF1A* (GeneID: 6927), a transcription factor required for the expression of several liver-specific genes, which is involved in glucose regulation and maturity onset diabetes of the young type 3. The F_0 wild boar in the W × M family was an *AG* heterozygote at the locus *358A>G* (for genotyping of the SNP see Appendix S1) and the four F_0 Meishan sows were *AA* homozygotes. The SNP was associated ($P < 0.05$, q = 0.0148–0.0794) with fat deposition, muscling, growth and fattening and other traits, but not with fat-to-meat ratio (Table S5). Most of the traits had *q* values lower than 0.05. Variation in the locus explained 1.6–3.9% of PV in F_2 animals. Although homozygous *AA* and *GG* genotypes did not differ significantly for fat deposition, muscling, growth and other traits, the *AG* heterozygotes had significantly higher (0.25–0.44 SD) values for the aforementioned traits when compared with the average of both homozygotes. Similarly, human individuals homozygous for either of two SNP variants in the *NAMPT* gene promoter region have lower fasting plasma insulin levels (Bailey *et al.* 2006).

Association analysis of haplotypes composed of SNPs *358A>G* and *669T>C* (dam phase/sire phase) showed that genetic variation in the *NAMPT* gene affects, in descending order, muscling, growth rate, fat deposition and meat quality (Tables 1 and S6). Compared with the single-locus association analyses, haplotypes accounted for a higher proportion of PV for muscling, growth, fat deposition, fat-to-meat ratio, and meat quality traits and revealed a higher degree of dominance. The double heterozygous animals by haplotype (genotypes of grandparents were used to determine grandparental origin information) (*AT*/*GC* + *GC*/*AT*) had 0.72, 0.68, 0.56 and 0.22 SD higher weight of loin and neck meat, live weight, fat area on *m. longissimus lumborum et thoracis* at 13th/14th rib and fat-to-meat ratio respectively and 0.44 and 0.30 SD lower cooling loss and pH_U in *m. longissimus lumborum et thoracis* 24 respectively when

Table 1 Association of haplotypes *FN392209:g.358A>G* and *AM999341:g.669T>C* within the porcine *NAMPT* gene and the most significant influenced traits in wild boar × Meishan (W × M) F_2 animals for growth, muscling, fat deposition and meat quality. (For each haplotype and trait, the least square mean with SE is given using GLM procedure.).

		Trait					
Haplotype ♀/♂	*n*	Loin and neck meat weight (kg) (PV% = 6.93, $P = 0.0002^{***}$, $q = 0.0056^{***}$)	Live weight at slaughter (kg) (PV% = 6.28, $P = 0.0004^{***}$, $q = 0.0056^{***}$)	Fat area on m. l. l. t. at 13th/14th rib (cm^2) (PV% = 5.19, $P = 0.0016^{**}$, $q = 0.0109^{**}$)	Fat-to-meat ratio (PV% = 4.89, $P = 0.0024^{**}$, $q = 0.0135^{**}$)	pH in m. l. l. t. 24 h post-mortem (PV% = 2.82, $P = 0.0293^{*}$, $q = 0.0695^{*}$)	Cooling loss (%) (PV% = 3.1, $P = 0.0202^{*}$, $q = 0.0568^{*}$)
AT/AT	44	$3.53 \pm 0.11^{A,C,a,c}$	$65.33 \pm 2.08^{A,B,E,a}$	$21.65 \pm 0.97^{A,B,C,b,c}$	$1.16 \pm 0.05^{A,B,a}$	$5.58 \pm 0.01^{A,B,a,b}$	$2.06 \pm 0.12^{A,B,a}$
AT/AC	43	$3.88 \pm 0.10^{a,b}$	$73.89 \pm 2.05^{A,C,c}$	$26.47 \pm 0.95^{A,D,a}$	$1.40 \pm 0.05^{A,C,b}$	$5.52 \pm 0.01^{A,C}$	$1.46 \pm 0.12^{A,b,c,d,e}$
AT/GC	77	$4.02 \pm 0.08^{A,B,E,F}$	$74.77 \pm 1.60^{B,D,G}$	$25.74 \pm 0.75^{B,f,e}$	$1.31 \pm 0.04^{D,a}$	$5.53 \pm 0.01^{B,c}$	1.63 ± 0.09^{B}
AC/AT	26	$3.49 \pm 0.14^{B,D,b,d}$	$64.15 \pm 2.83^{C,D,F,b}$	$22.41 \pm 1.32^{a,d,e}$	$1.16 \pm 0.07^{C,E}$	5.57 ± 0.02	1.96 ± 0.16^{b}
AC/AC	19	3.75 ± 0.16	70.39 ± 3.11	25.35 ± 1.45^{b}	1.32 ± 0.08^{c}	5.54 ± 0.02	1.88 ± 0.18^{c}
AC/GC	52	$3.86 \pm 0.10^{c,d}$	$72.29 \pm 1.96^{a,b}$	24.96 ± 0.92^{c}	$1.26 \pm 0.05^{F,b}$	5.54 ± 0.01^{a}	1.83 ± 0.11^{d}
GC/AT	18	$4.08 \pm 0.16^{C,D,e,f}$	$76.61 \pm 3.24^{E,F,d}$	24.73 ± 1.51	1.23 ± 0.08^{G}	$5.59 \pm 0.02^{C,c,d}$	1.62 ± 0.19^{a}
GC/AC	11	$3.47 \pm 0.20^{E,f}$	69.17 ± 3.96	$27.83 \pm 1.85^{C,d,g}$	$1.60 \pm 0.10^{B,D,E,F,G,H,c}$	5.53 ± 0.03	1.65 ± 0.23
GC/GC	44	$3.61 \pm 0.10^{F,e}$	$67.62 \pm 2.06^{G,c,d}$	$22.84 \pm 0.96^{D,f,g}$	1.28 ± 0.05^{H}	$5.54 \pm 0.01^{b,d}$	1.84 ± 0.12^{e}

$P = P$ value of the F test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; PV% = the proportion of phenotypic variance that is explained by the *NAMPT* haplotypes; q = P value adjusted for multiple testing (Benjamini & Hochberg 1995); m. l. l. t. = *m. longissimus lumborum et thoracis*; matching capital letters indicate differences significant at level $P < 0.01$; matching small letters indicate differences are significant at level $P < 0.05$. Haplotypes for double homozygotes and heterozygotes using phase information deduced from grandparents are given in bold (*AT/AT* = both haplotypes from grand maternal origin, first from dam and second from sire of the F_2 animals; *GC/GC* = both haplotypes from grand paternal origin; *AT/GC* and *GC/AT* = haplotypes originated from both grandparents).

compared with the mean of the double homozygous (*AT*/*AT* and *GC*/*GC*) animals.

In the LCE population, frequency of the *C* allele at the locus *669T>C* was 0.61 (for genotyping of the SNP see Appendix S1). In this population, as in other European Meishan-cross lines, the level of LD is expected to be higher than that in purebred Chinese breeds, extending up to 0.05 cM, although still lower than in most European breeds, extending up 2 cM. Populations with a shorter extent of LD are more suitable for fine-mapping of genes responsible for phenotypic traits (Amaral *et al.* 2008). In the LCE population, the locus *669T>C* was associated ($P < 0.05$) with ham weight, meat colour and fatty acid composition (Table 2). Animals with the *TT* genotype had lower weight of ham and lower values for Minolta *a** (redness) compared with animals with *CT* ($P < 0.01$) and *CC* ($P < 0.05$) genotypes. Values for Minolta *b** (yellowness) were significantly higher in the *CC* genotypes. The *TC* genotype was associated with lower palmitic fatty acid percentage in backfat (C16:0) compared with the *TT* genotype and higher linoleic (C18:2) fatty acid content compared with the *TT* and *CC* genotypes. The *669T>C* locus explained 0.9–4.7% of total PV in associated traits. In the LCE population, association analysis of SNP *358A>G* was not performed, as the allele *G* is nearly fixed in the founder animals (Landrace $p_G = 0.92$, Large White $p_G = 0.93$, Meishan that belongs to Taihu pigs $p_G = 1$).

Results obtained in the LCE population confirmed the findings that the locus affects muscling and meat quality in the same direction as in W × M family. Lower significance and probably limited biological relevance of associations in the LCE compared with the W × M could be attributable either to erosion of LD between the SNP and a causative mutation or to the different genetic backgrounds (wild boar vs. Landrace and Large White). Despite the different statistical models used, it can be concluded that neither fat-to-meat ratio in the W × M nor backfat depth with carcass weight as covariate in the LCE was affected by the SNP *669T>C*. Contrary to that, haplotypes were significantly associated with fat-to-meat ratio in the W × M, which could not be validated in the LCE for the aforementioned reason.

A previous whole genome scan in the W × M family (Čepica *et al.* 2003) revealed QTL only for weight of liver, live weight at slaughter and fat-to-meat ratio at positions 64, 78 and 148 cM respectively, while *NAMPT* mapped to position 121 cM on this map. Discrepancy between the results of QTL and association analysis is expected, as the SNP *669T>C* segregated in both founder populations and SNP*358A>G* segregated in the F_0 wild boar male. In different pig populations, the *NAMPT* gene is located in the 95% confidence intervals of QTL for backfat, growth, body composition, feed intake, fatty acid composition and loin pH_U (PigQTLdb, Hu & Reecy 2007).

In both populations the locus *669T>C* and haplotypes *358A>G* and *669T>C*, but not SNP *358A>G*, were

Table 2 Significant associations of the *AM999341:g.669T>C* locus within the *NAMPT* gene with carcass composition and meat quality traits in Landrace × Chinese-European synthetic population. (For each genotype and trait, the least square mean with SE is given using GLM procedure.)

	F test				LSmeans ± SE			*P* (*t* test)		
Population/Trait	*F* ratio	*P* value	*q*	PV (%)	*TT*	*TC*	*CC*	*TT* vs. *TC*	*TC* vs. *CC*	*TT* vs. *CC*
Growth and carcass traits					*n* = 172	*n* = 203	*n* = 72			
Ham weight (kg)	3.06	0.0479	0.1629	0.93	11.59 ± 0.03	11.70 ± 0.03	11.70 ± 0.05	0.0201*	0.9823	0.0891
Meat and fat quality traits					*n* = 83	*n* = 74	*n* = 23			
Minolta *a**	4.93	0.0082**	0.0697	4.25	5.58 ± 0.15	6.18 ± 0.16	6.34 ± 0.28	0.0070**	0.6226	0.0199*
Minolta *b**	5.32	0.0057**	0.0697	4.65	11.02 ± 0.14	11.30 ± 0.15	12.03 ± 0.27	0.1879	0.0215*	0.0014**
C16:0, %	3.56	0.0296*	0.1258	1.53	23.69 ± 0.08	23.43 ± 0.07	23.69 ± 0.13	0.0130*	0.0785	0.9641
C18:2, %	4.31	0.0142*	0.0805	1.97	9.55 ± 0.05	9.72 ± 0.05	9.46 ± 0.09	0.0243*	0.0112*	0.3658

P = *P* value of the *F* test; **P* < 0.05; ***P* < 0.01; *q* = is *P* value adjusted for multiple testing (Benjamini & Hochberg 1995); PV (%) = the proportion of phenotypic variance that is explained by the genotypes of the *NAMPT* polymorphism.
Apart from the significantly associated traits, the following traits were tested: backfat 160d, backfat 187d, carcass backfat P2, carcass backfat GM, live weight at slaughter, carcass weight hot, shoulder weight, Minolta *L**, C18:0, C18:1.

associated with meat quality (pH_U, cooling loss and meat colour). The pH_U of pork as a major quality determinant is highly correlated with glycolytic potential, drip loss and meat colour (Hamilton *et al.* 2003; Scheffler & Gerrard 2007). Hypothetically, differences in pH_U between the *669T>C* genotypes could be caused either by differences in glycolytic potential or by differences in availability of NADH for formation of lactate from pyruvate.

NAMPT plays a critical role in the regulation of glucose-stimulated insulin secretion and NAMPT-mediated NAD biosynthesis, which affects, through the NAD-dependent deacetylase SIRT2, a range of basic biological processes including the pace of metabolism, gluconeogenesis, glycolysis, cholesterol metabolism, lipolysis, free fatty acid metabolism and insulin secretion (Garten *et al.* 2009; Imai 2009). Results of genetic association studies in man suggest a possible role for *NAMPT* gene polymorphisms in insulin, glucose and obesity-related phenotypes (Bailey *et al.* 2006; McKenzie 2008; Blakemore *et al.* 2009).

The SNPs detected in our work are expected to be in LD with a causative mutation affecting energy metabolism as a whole rather than fat metabolism alone. Theoretically, the associated effects can be caused by variation in the *NAMPT* gene itself. However, because no differences in the coding sequence between wild boar and Meishan pigs (see Appendix S1) and only a limited number of SNPs within the porcine *NAMPT* gene have been studied, further research on heterogeneity of the gene regulatory regions is needed to elucidate whether the causative mutation is within the gene or in its vicinity in the chromosome region homologous to human chromosome 7q22.2. The results for the studied SNPs need further replication in other populations.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Description of porcine *NAMPT* primers.

Table S2 Allele frequencies at loci *AM999341:g.669T>C* and *FN392209:g.358A>G* of the *NAMPT* gene in various populations.

Table S3 Traits used for association analysis of *NAMPT* genotypes/haplotypes in wild boar × Meishan (W × M) F_2 family.

Table S4 Significant associations of the *AM999341:g.669T>C* locus within the *NAMPT* gene with carcass composition and meat quality traits in wild boar × Meishan (W × M) F_2 animals. (For each genotype and trait, the least square mean with SE is given using GLM procedure.)

Table S5 Significant associations of the *FN392209:g.358A>G* locus within the *NAMPT* promoter with carcass composition traits in wild boar × Meishan F_2 animals.

Table S6 Significant associations of haplotypes including *FN392209:g.358A>G* and *AM999341:g.669T>C* within the porcine *NAMPT* gene and traits for growth, muscling, fat deposition and meat quality in wild boar × Meishan F_2 animals.

Appendix S1 Materials and methods.

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